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Structural Characterisation of Widespread Polyunsaturated Isoprenoid Biomarkers: A C_{25} Triene, Tetraene and Pentaene from the Diatom *Haslea ostrearia* Simonsen.

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Abstract: Three highly branched isoprenoid (HBI) polyenes have been isolated from the diatom *Haslea ostrearia* and characterised by nmr spectroscopy.

The primary attributes of acyclic isoprenoid hydrocarbons as geochemical biomarkers, a role in which they are commonly used, are the distinctive structures and stereochemistries inherited during biosynthesis. [1] However, rigorous structural characterisation is often precluded by the low concentration found in sediments and the complexities of the mixtures therein. Under such circumstances, large amounts of sediments and time-consuming, tedious isolation steps are necessary. Such drawbacks have to date proved sufficient to confine complete structural characterisation of the numerous widespread and abundant unsaturated highly branched isoprenoid (HBI) hydrocarbons (Figure 1) to six monoenes [2] and a diene. [3] These compounds occur with 0 to 7 degrees of unsaturation and are often the most abundant hydrocarbons in recent sediments. [2-10]

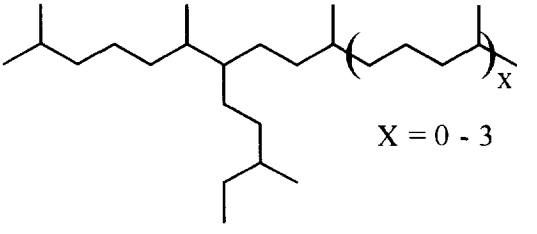

Figure 1. Parent structures for HBI hydrocarbons: C_{20} (**1**), C_{25} (**2**), C_{30} (**3**), C_{35} (**4**)

Fortunately, the recent identification of a biological source of the sedimentary HBIs, the diatomaceous alga, *Haslea ostrearia*, [9] reveals an alternative source for their isolation. Indeed, by culturing *Haslea ostrearia* on a large scale (500 litre tanks) in natural conditions, we have been successful in isolating and characterising the structures of three HBI polyenes using nmr spectroscopy. This represents the first example of a structural determination study of HBIs isolated from a source organism.

A single batch of *Haslea ostrearia* was cultured to obtain a dense population of algae similar to that observed in natural blooms contained in oyster ponds.[11] After 7 days, the algae were concentrated to *ca* 37 g wet weight by centrifugation. A total hexane extract (THE, 198 mg) rather than a total organic extract (TOE) was obtained from the resulting paste in order to both fully isolate the HBI alkenes and at the same time, minimise the extraction of pigments and more polar lipids. Analysis of the THE by GC and GC-MS revealed that the three major components of this extract were a C_{25} triene ($C_{25:3}$), tetraene ($C_{25:4}$), and pentaene ($C_{25:5}$).[9] Isolation of the individual HBIs was achieved using column chromatography (SiO_2 / hexane). Quantification of the HBIs is summarised in Table 1.

	$C_{25:3}$	$C_{25:4}$	$C_{25:5}$
Isolate / mg	5.1	18.9	2.8
% THE	2.5	9.4	1.4
% HBI	19.0	70.5	10.5
[HBI] / fg cell^{-1}	290	822	164

Table 1. Quantities of HBIs isolated from *Haslea ostrearia*

Each of the HBIs was hydrogenated ($PtO_2.2H_2O$, hexane, H_2) to give a single compound which co-chromatographed (GC) with authentic **2** on 2 phases (OV1 and DB5).[4] Once the carbon skeleton of each HBI had been verified, the positions and stereochemistries of the double bonds were determined using ^1H and ^{13}C nmr spectroscopy including 2-D (1-3 bond correlations) and homonuclear decoupling experiments.† The structures of the triene (5), tetraene (6) and pentaene (7) are shown in Figure 2.

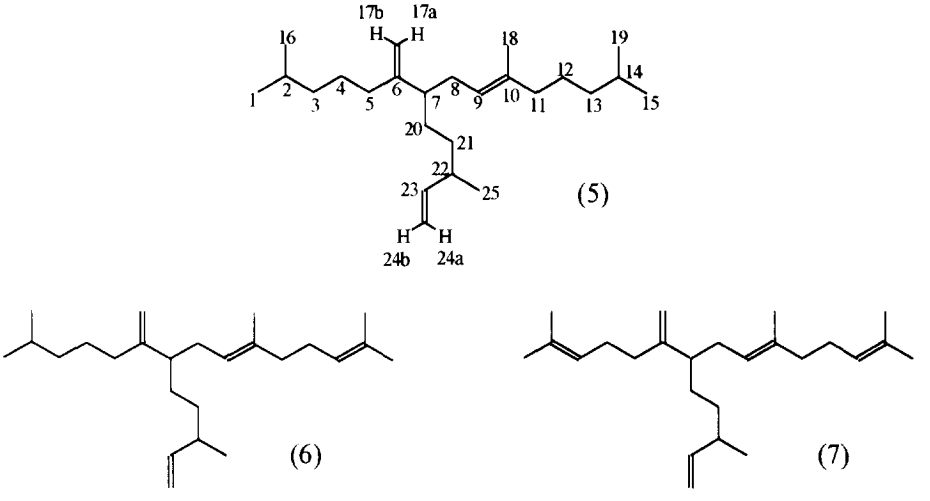

Figure 2. Structures of C_{25} triene (**5**), tetraene (**6**) and pentaene (**7**)

The common structural features of the three HBIs are (i) a vinyl group (C23-C24) identified by an ABMX spin system in the ^{1}H nmr spectrum, (ii) a methylenic double bond (C6-C17) identified by ^{1}H nmr and located *via* a longe range correlation ($^3J_{CH}$) between H-17 and C7 and (iii) a trisubstituted double bond (C9-C10) confirmed by all nmr methods. Relative to the triene (**3**), the tetraene (**4**) and pentaene (**5**) incorporate an additional one and two isobutenyl moieties respectively. For the most abundant HBI (**6**), an alternative position for the single isobutenyl function can be envisaged at C2-C3. This alternative structure can be discounted for the following reasons. A long range coupling ($^3J_{CH}$) is observed between H-17 and a methylenic carbon which allows an assignment for C5 to be made. An assignment for H-5 follows from the $^1J_{CH}$ correlation. H-5 also shows a weak $^2J_{CH}$ correlation to a methylenic carbon (C4) which in turn ($^1J_{CH}$) demonstrates that H-4 is non-allylic (δ = 1.4 ppm). More convincingly, irradiation of H-5 at 1.87 ppm, affects neither of the two other allylic proton resonances at 1.96 and 2.04 ppm, while a broad multiplet at 1.4 ppm is decoupled. Taken together, these observations demonstrate that H-4 is non-allylic and therefore the isobutenyl double bond cannot be located in the C2-C3 position. The identification of the C13-C14 double bond also fixes the position of the trisubstituted double bond (C9-C10). The alternative isomer (C10-C11) would result in a 'double allylic' pair of protons (H-12) which are not observed.

Finally, the spectra of all three HBIs contain several resonances which 'double-up' in appearance. This feature is particularly clear for the vinylic proton (H-23) and carbons C5-C8 and C20-C25. Since all three HBIs contain two chiral centres (C7 and C22), with the double-up resonances arising due to the carbons closest to these, we propose that these observations can be explained by invoking the presence of a mixture of diastereoisomers. An alternative explanation for these spectral observations involving E/Z isomerism about C9-C10 can be rejected since the resonances due to C9-C15 and C18,19 do not appear as two sets as would be expected for geometrical isomers. Further, although these diastereoisomers are observed readily by nmr, chromatographic separation (GC) does not take place on the phases used (*vide infra*). To date, we have not attempted to separate the diastereoisomers by chiral chromatography.

We acknowledge Dr T.M. Peakman (University of Bristol) for obtaining 400 MHz nmr spectra.

† NMR data for $C_{25:3}$, $C_{25:4}$ and $C_{25:5}$ at 270 MHz for ^{1}H in $CDCl_3$. ^{1}H and ^{13}C chemical shifts are in ppm using $CDCl_3$ as an internal reference (7.25 ppm from residual $CHCl_3$ for ^{1}H and 77.0 ppm for ^{13}C respectively). Numbering shown in displayed formulae. Chemical shifts labelled * indicate the 'doubling-up' of resonances due to the presence of diastereoisomers. (**5**): ^{1}H: δ 5.67, 5.65* (ddd, H-23), 5.05 (t, J = 6.6 Hz, H-9), 4.92 (br,d, H-24a), 4.88 (br,d, H-24b), 4.74 (br,d, H-17b), 4.70 (br s, H-17a), 2.02 (m, H-8,22), 1.89 (m, H-5,7,11), 1.56 (s, H-18), 0.86 (d, J = 6.6 Hz, H1,16,15,19); ^{13}C, DEPT: δ 152.5* (C-6), 145.0, 144.9* (C-23), 135.6 (C-10), 123.1 (C-9), 112.4, 112.2* (C-24), 108.6* (C-17), 46.8 (C-7), 40.0 (C-11), 39.0 (C-3), 38.6 (C-13), 38.0, 37.9* (C-22), 34.4 (C-21), 33.7, 33.6* (C-5), 32.9, 32.8* (C-8), 30.9, 30.8*

(C-20), 28.0 (C-14), 27.9 (C-2), 25.7 (C-12), 25.6 (C-4), 22.6 (C-1,15,16,19), 20.4, 20.0* (C-25), 16.1 (C-18). (**6**): ^{1}H: δ 5.67, 5.65* (ddd, 1H, J_{trans}= 17.5, J_{cis} = 11.5, J_{vic} = 7.5 Hz, H-23), 5.08, 5.07 (br,m, 2H, H-9,13), 4.92 (br,d, 1H, H-24a), 4.88 (br,d, 1H, H-24b), 4.74 (br,d, 1H, H-17b), 4.70 (br,s, 1H, H-17a), 2.04 (m, 5H, H-8,12,22), 1.96 (m, 3H, H-7,11), 1.87 (t, 2H, J = 7.5 Hz, H-5), 1.67 (s, 3H, H-15), 1.59 (s, 3H, H-19), 1.57 (s, 3H, H-18), 0.95 (d, 3H, J = 6.9 Hz, H-25), 0.87 (d, 6H J = 6.6 Hz, H-1,16); ^{13}C, DEPT, ^{13}C-^{1}H: δ 152.5, 152.4* (C-6), 145.0, 144.8* (C-23), 135.2 (C-10), 131.2 (C-14), 124.4 (C-13), 123.3 (C-9), 112.4, 112.2* (C-24), 108.6* (C17), 46.8 (C-7), 39.8 (C-11), 39.0 (C-3), 38.0, 37.9* (C-22), 34.3, 34.4* (C-21), 33.7, 33.6* (C-5), 32.8, 32.7* (C-8), 30.9, 30.8* (C-20), 27.9 (C-2), 26.7 (C-12), 25.7 (C-15), 25.6 (C-4), 22.6 (C-1,16), 20.4, 20.0* (C-25), 17.7 (C-19), 16.2 (C-18). (**7**): ^{1}H: δ 5.67, 5.65* (ddd, H-23), 5.09 (m, H-3,9,12), 4.92 (d, J = 17.5 Hz, H-24a), 4.88 (d, J = 9.6 Hz, H-24b), 4.76 (d, J = 1.7 Hz, H-17b), 4.72 (br s, H-17a), 1.67 (s, H-1,15), 1.6, 1.59 (s, H-16,19), 1.57 (s, H-18), 0.95 (d, J = 6.9 Hz, H-25); ^{13}C, DEPT; δ 152.1 (C-6), 145.0, 144.8* (C-23), 135.3 (C-10), 131.3, 131.2 (C-2,14), 124.5, 124.4 (C-3,13), 123.3 (C-9), 112.4, 112.2* (C-24), 108.9 (C-17), 47.0 (C-7), 39.8 (C-11), 37.9 (C-22), 34.3 (C-21), 33.1 (C-5), 32.8, 32.7* (C-8), 30.8, 30.7* (C-20), 26.7 (C-12), 26.4 (C-4), 25.7 (C-1,15), 20.4, 20.0* (C-25), 17.7 (C-16,19), 16.2 (C-18).

‡ The doubled-up resonances observed in the ^{13}C spectrum of (**6**) have the same chemical shifts at 67.8 and 100.4 MHz

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